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Research

Assessment of bisphenol A levels in preschool children-Results of a human biomonitoring study in Ankara, Turkey

Short title: Bisphenol A levels in preschool children

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Abstract
Objective: There is general concern regarding environmental chemical exposures and the impact it may have on human health, which is particularly important for vulnerable populations such as infants and children in critical periods of development. Bisphenol A (BPA) is an endocrine disrupting chemical used worldwide over the last 30 years in many consumer products that play an important role in our daily life. The aim of this study was to evaluate the exposure of Turkish preschool children to BPA.
Methods: This study conducted a preliminary investigation of BPA in urine collected from 3-6 year old children (mean age: 4.50±1.26) living in Ankara. For this purpose, after spot urine samples were taken from preschool children (n=125; **Males** n= 70, **Females** n=55), free BPA, β-D-glucuronide (BPA-GLU) and total BPA were determined using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).
Results: Total BPA was detected in 76.8% of children from Ankara city, with concentrations ranging from <LOQ to 18.36 µg/g creatinine. Total BPA levels were not statistically different between boys (1.26 µg/g creatinine) and girls (2.24 µg/g creatinine) ($p>0.05$). The estimated daily BPA intake in this study is substantially lower than the European Food Safety Authority derived tolerable Daily intake (TDI) of 4 µg/kg BW/day.
Conclusion: This study is an important contribution to the limited information about childhood exposure to BPA.
Keywords: BPA, urine, children, LC-MS/MS, Turkey

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Introduction

There is general concern regarding environmental chemical exposure and its impact on human health, but this is particularly important for vulnerable populations such as infants and children in sensitive periods of development. In 1997 the leaders of the G8 countries stated, "*We acknowledge that, throughout the world, children face significant threats to health from an array of environmental hazards. The protection of human health remains a fundamental objective of environmental policies to achieve sustainable development. We increasingly understand that the health and well-being of our families depends upon a clean and healthy environment. Nowhere is this more true than in the case of children, who are particularly vulnerable to pollution*". In addition, one of the biggest concerns of the World Health Organization (WHO) for children is exposure to chemicals during the intrauterine and childhood periods and associated health problems that arise later in life (1). In recent years, chemical fluctuations of endocrine disruptors (EDs) have been implicated in the incidence of many diseases and disorders.

Bisphenol A (BPA), with approximately 3.6 million tons annual global production (2), is an ED. The United States Environmental Protection Agency (EPA) estimates that more than 400,000 kilograms of BPA are leached into the environment every year (3). Because of the widespread use of BPA, over 90% of tested humans have detectable BPA, with the highest levels found in infants and children (4).

Childhood exposure to BPA occurs through specific exposure routes including mouthing, food intake, and the use of BPA-containing products, inhalation, dermal contact, and ingestion. Children are more susceptible to chemicals such as BPA than the general population due to their rapid development and increased food intake per kg body weight (1). BPA exposure has been linked to a range of advers human health outcomes including decreased fertility, behavioural effects, disruption of endocrine function, altered development, and increased metabolic diseases. For example, relationships between BPA exposure and altered neurobehavioral outcomes (hyperactivity, attention problems, anxiety, and depression) in children have been reported by several human studies (5-10) . Following the demonstration of a wide variety of adverse effects associated with BPA exposure in humans and laboratory animals over the last two decades, the Canadian Ministry of Health banned the import and marketing of infant feeding bottles made of polycarbonate in 2008. In 2011, the European Union banned BPA use in the production of polycarbonate baby bottles and prohibited the sale and import of BPA-containing products that come in contact with food for children aged 0–3 (11). The same restrictions have applied in Turkey since 2011.

Numerous studies estimate exposures to BPA using urinary biomonitoring. Most have focused on adults from the different societies to quantify human exposure to BPA. These studies have shown large variations between participants and studies, but very limited data are available for young children. To our knowledge, only few data are available regarding human exposure to BPA in Turkey. The primary aim of this study was to quantify exposure of preschool children to BPA.

Materials and Methods

Chemicals and reagents

All chemicals used were of analytical grade. BPA and creatinine standards were purchased from Sigma-Aldrich (St Louis, MO, USA). The isotope-labeled internal standards $^{13}C_{12}$-BPA (99 %), creatinine-d3 and $^{13}C_{12}$-bisphenol A β–D-glucuronide ($^{13}C_{12}$-BPA-GLU) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). HPLC-grade methanol and acetonitrile (KGaA, Darmstadt, Germany) were used. Ammonium acetate was obtained from J. T. Baker (Phillipsburg, NJ, USA). Stock solutions of the BPA (100 ng/ml) and BPA-GLU (1000 ng/mL) were prepared in methanol and were stored in the dark at −20 °C. Deionized water (18.2 MΩ) treated with the Millipore (Simplicity, 185) Milli-Q water purification system (Elga Labwater Veolia, Anthony, France) was used for all aqueous solutions.

Study population

Urine samples were collected from preschool children (3-6 years old) between November 2015 and May 2016. Four day-care centers in Ankara, Turkey participated and 125 children were recruited for this study (55 female, 4.42±1.09 years old ; 70 male, 4.56±1.39 years old). Table 1 presents the distribution of the main characteristics of the study populations. Each parent completed a questionnaire about their children's dietary habits, exposure to BPA in their daily life, home, and school environment, medical history, weight, and height. The study was designed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Approval for the study protocol was obtained from the Ethics Commission of the Mersin University Clinical Research Ethical Committees in Mersin, Turkey (document number 12.02.2015/37). Written informed consents were obtained from all parents of individual participants.

Each child provided a single spot urine sample collected in 125 mL glass (PTFE_lined) screw cap culture tube that had been previously cleaned with hexane and divided into aliquots. Urine samples from healthy children were collected in the evening before a meal to determine the spot urine concentrations of both free BPA and BPA-GLU. The samples were kept cool until transportation to the study center and were immediately frozen -20 °C until analysis.

Sample preparation

Sample preparation, chromatographic, and mass spectrometric conditions were used as described previously (12). Briefly, $^{13}C_{12}$-BPA was used as a stable internal standard and added to the samples at the beginning of the extraction. The BPA and BPA-GLU in urine samples (500 µL) were purified by protein precipitation/dilution with 500 µL of acetonitrile and 50 µL of $^{13}C_{12}$-BPA and $^{13}C_{12}$-BPA-GLU. After protein precipitation samples were centrifuged at 2250 rpm at 25 °C for 10 min. Total BPA values were calculated as reported previously by Battal et al. (12). All other analytical data such as QA/QC assurance, matrix effets, data repeatability, etc, reported our previous study as well (12).

Instrumental analysis

Identification and quantification of the free BPA and BPA-GLU were performed with an Agilent 1200 Series 6460 triple quadrupole mass spectrometry with Jet-Stream atmospheric pressure electrospray ionization source and Mass Hunter data acquisition / Quantitation software. The HPLC system was equipped with a binary pump, vacuum degasser, low carryover autosampler and thermoregulated column compartment. Twenty microliters of the extract was injected onto an Agilent Pursuit 3 Pentaflouraphenyl Propyl (PFP) column (100×3.0 mm, 3 µm particle size). The mobile phases A and B consisted of 2 mM ammonium acetate in water and methanol respectively. The limits of detection (LOD) for free BPA and BPA-GLU were 0.03 ng/ml, and 0.10 ng/ml and the limits of quantification (LOQ) were 0.08 ng/ml and 0.33 ng/ml respectively. The tandem mass spectrometry (MS-MS) was operated with negative electrospray ionization in the selected reaction monitoring (SRM) mode. Nitrogen was used as both curtain and collision gas. The monitored quantifier SRM transitions were 227.1>132.8 for free BPA, 403.1>113.1 for BPAG, and 239.2>224.1 for $^{13}C_{12}$-BPA (internal standard).

Creatinine analysis in urine

Both the free BPA and BPA-GLU values obtained in this study were corrected for creatinine. To assess the impact of creatinine adjustment on the total variance of spot urine samples, urine creatinine levels were analyzed using a modified method developed and validated for creatinine analysis by Park et al. (13). Briefly, an aliquot of urine (10 µL) was diluted with milli-Q water (1000-fold) and 100 µL (5 mg/L) of creatinine-d3 (internal standard, 5 mg/mL) was added. Creatinine was analyzed with LC−MS/MS in electrospray positive ionization mode and the SRM transitions monitored were 114.1>86.1 for creatinine and 117.2>89.2 for creatinine-d3. One microliter of the extract was injected onto an Agilent Zorbax SB-C18 chromatographic column (3 X 50 mm, 3.5 µm particle sizes). The mobile phases A (water) and B (methanol) both had 2 mM ammonium acetate. The analysis for creatinine was achieved using isocratic conditions (80%B).

Estimated daily intake (EDI) calculation

To understand the magnitude of BPA exposure by children, EDI (estimated daily intake) was calculated based on the assumption of urine excretion volumes of 0.4 L (ages 3–4 years) and 0.5 L (ages 5-6 years) for 24 h for children (14). The daily exposure doses of BPA were estimated using the following equation:

$$\text{Estimated daily intake (EDI) (µg/kg bw/day)} = \frac{\text{Urinary BPA concentration (µg/L)} \times \text{Urinary Output (L/day)}}{\text{Body weight (kg)}}$$

Statistical analysis

The statistical evaluations of the data were performed with SPSS version 11.5 for Windows. Data were summarized as minimum, maximum, median, mean, geometric mean, and standard deviation for total and each group. The normality of the data distribution was assessed with the Shapiro-Wilk test. The Mann–Whitney U test was used for multiple comparisons between groups. p values equal to or less than 0.05 were accepted as statistically significant.

Results

In this study, free BPA and glucuronide conjugate of BPA (BPA-GLU) were measured in preschool children who live in Ankara. Urinary total BPA concentrations (adjusted for creatinine) in **females** and **males** are presented in Table 2. Total BPA was determined in 76.8% of the analyzed urine samples and BPA concentrations were equal to or above the LOQ of

0.08 ng/ml. Total urinary concentrations of BPA in Turkish preschool children ranged from LOQ-18.36 µg/g creatinine, with a mean concentration of 1.79 µg/g creatinine. The mean concentrations of total BPA in **females** and males groups were 2.24 µg/g creatinine and 1.26 µg/g creatinine, respectively, and there was not a statistically significant difference (p= 0.2022). On the other hand, when age groups were taken into consideration (<4 years and >4 years), the mean BPA values of **<4** years-old **females** were statistically higher than the **males** of the same age group were **(p=0.005)** (Table 3, Fig.1).
For positive samples (values > LOQ) daily intakes ranged from 7 ng/kg bw/day to 2.916 ng/kg bw/day. The EDI for the preschool children was calculated as 35 ng/kg **bw**/day (geometric mean [GM]) in this study. The mean EDI values were lower for the **males** group than **females** (Table 4), but this difference was not statistically significant (p>0.05). For risk assessment, the European Food Safety Authority (15) and the US Environmental Protection Agency (16) recommened 50 µg/kg bw/day dose for the tolerable daily intake (TDI) and reference dose (RfD) for BPA exposure. The European Commission estimated the daily intake of BPA to be 0.4 µg/kg bw/day for adults, 1.2 µg/kg bw/day for children between 4 and 6 years, and 1.6 µg/kg bw/day for infants in EU countries (17). The EFSA revised the TDI for BPA to 4 µg/kg bw/day in January 2015 (18). The GM and 95th percentile daily intakes of BPA determinated in both age groups and gender groups in this study were much lower than the guidelines established by the EFSA and US EPA (Table 4). This indicates that Turkish preschool childrens have a safe level of BPA exposure.

Discussion

BPA is a high trade volume chemical because it is widely used in many consumer products and exposure is almost inevitable in daily life. In addition to being the first study to evaluate the BPA exposure in preschool children in Turkey, the results of this study are important to supply basic data on BPA concentrations in the human population in Turkey. Studies assessing BPA exposure show that because of a dramatic increase in the use of BPA-containing products in daily life, BPA and its metabolites are present at detectable levels in nearly every person's blood, tissue, and urine. In order to assess the exposure of BPA in humans, measurement of their urinary concentration of free species and their conjugates is essential (19,20). BPA in biological samples is observed as both free BPA and conjugated BPA. Among conjugated BPAs, BPA-GLU is a sufficiently specific and stable compound that can be regarded as a biomarker to evaluate BPA exposure. Varying levels of BPA and BPA-GLU are detected in urine samples depending on nutrition and lifestyle.
Although there is a general concern about possible effects of exposure to environmental chemicals on human health, these concerns are especially important for susceptible communities such as babies and children, which are in critical stages of their development. One of the biggest concerns of the WHO regarding infants is health problems that will show up later in life because of exposure to chemicals during the intrauterine and childhood periods. In particular, endocrine disrupting chemicals make important alterations in cellular pathways that provide a basis for these diseases (1). Hormones are the chemicals that regulate physiological homeostasis and functions of our body. These regimens are carried out in very small doses at the "picogram" level. Therefore, as a results of continuous exposure to EDs such as BPA, minor changes in hormone levels may cause major changes in our bodies and for us (1).
BPA is an endocrine disrupting compound (21) and plays an important role in daily life due to its widespread use worldwide in many consumer products over the past 30 years all over the world (toys, baby bottles, plastic storages, heating containers for food and beverages, the lining of metal cans, medical equipment, consumer electronics, dental sealants etc.). A recent hypothesis states that BPA exposure may lead to many health risks, particularly obesity (22) and poor reproductive health (23). Because exposure to this compound during a critical period such as childhood will provide a basis for exposure-related health problems, it is vital to determine the extent of BPA exposure in childhood both for the health of the individual and society.
Numerous biomonitoring studies of children to quantifify childhood exposure to BPA during the last decade from different societies and different age groups have reported large variations between participants and studies. However, there are a limited number of studies on BPA exposure levels in preschool children. Huang and coworkers (24) calculated the average global EDI of BPA for children based on the results of studies for 2-17 year old children from 18 nations between 2000 and 2016. The average global EDI for the children was 60.08 ng/kg bw/day in their study. In our study, we detected half of this value (35 ng/kg bw/day). Huang et al. (2017) (24) found that Taiwan had the highest estimated child BPA daily intake with 201.00 ng/kg bw/day, whereas Italy had the lowest with 15.34 ng/kg bw/day.
Due to anticipation that exposure of children might be particularly high, recent studies have determined the BPA exposure extent in preschool children in various countries. Some examples of these studies are summarized in Table 5, with a focus on studies assessing preschool children similar to our study. Studies showed that BPA levels decrease with increasing age in almost every society. For example, in a Health Measures Survey conducted in Canada between 2007 and 2011, the youngest study group, consisting of children aged 6-8, had the highest BPA level (6) (Table 5). Similarly, the 3–5 year age group (GM 3.55 µg/L) had a higher urinary BPA concentration than the 6–8 (GM 2.72 µg/L), 9-11 (GM 2.22 µg/L), and 12–14 (GM 2.42 µg/L) year age groups in the German Environmental Survey for Children (25). These results indicate that younger people, particularly infants and children below age 6, are subjected to higher exposure risk. Similar results were obtained in our study.
In this study, no significant associations between the consumption of various canned foods and beverages and BPA levels were found (p>0.05) (Table 6). Urinary BPA levels of children consuming their food in heated plastic containers were found higher, but it was not statistically significant (p>0.05). Dental materials made of BPA derivatives such as bisphenol-A- dimethacrylate and bisphenol-A-diglycidyl-dimethacrylate, have been used as an alternative to mercury amalgams in dentistry. Therefore, in this study, whether the children had white dental filling was also evaluated. Composite restorations were not associated with urinary BPA concentrations in our study (p > 0.05). Further, there were no statistical associations between BPA levels and the use of plastic materials and toys (p > 0.05).
A few studies determined the BPA exposure level of individuals in Turkey. In 2014, mean urinary BPA values were 0.61 µg/g creatinine in 200 people in Mersin city (26). On the other hand, BPA amounts were quantified for 26 female children aged 4-8 years having Idiopathic Central Precocious Puberty (ICPP) disease and 21 healthy controls. The average BPA concentration was 1.62 µg/g creatinine for the healthy group, whereas this value was 8.34 µg/g for the disease group (27). As

a result, the estrogenic effects of BPA may be an etiologic factor for ICPP. Similarly, a study was performed on newly diagnosed ICPP patients (n=42; age: 7.4 ± 0.68) and peripheral precocious puberty (PPP) patients (n=42 age; 7.4 ± 0.61) between August 2012 and July 2013 in Ankara. Urinary BPA levels were 10.63 µg/g creatinine for ICPP patients and 10.15 µg/g creatinine for PPP patients (28).

In a very recently completed study, BPA was detected in 100% of 40 maternal urine (GM; 0.12 µg/L), their 1–2-month-old infant urine (GM; 0.13 µg/L), and breast milk (GM; 0.12 µg/L). However, BPA concentrations of the specimens were found relatively lower as compared to previous studies (29). **In another recent study, urinary BPA levels of 50 children with type 1 diabetes mellitus and 50 healthy children (aged between 5 and 18 years) were measured using high-performance liquid chromatography (37). In this study, urinary BPA levels of children with type 1 diabetes mellitus and healthy children were determined as 27.71 ± 17.53 µg/g creatinine and 25.37 ± 17.89 µg/g creatinine respectively. These values are quite higher than the values found in our study and other studies on this subject. This may be due to the HPLC method used to determine urinary BPA levels. Since HPLC is not a low-precision chromatographic method for determining BPA levels, it is not currently preferred by researchers to determine low BPA levels in biological materials.**

Study Limitations

Present study is an important contribution to the limited information about exposure to BPA during childhood in Turkey. Although 125 children from Ankara were included in this study, this number is not sufficient for this type of biomonitoring study. However, our results might be evaluated as preliminary finding. In order to provide a better understanding of exposure to BPA studies on wider population are needed and daily exposure levels from different sources should be determined. It seems useful that future studies should focus on children living in different provinces and highly exposed to BPA during the critical periods of the life span.

Conclusions

From a global perspective, the average BPA exposure for children is much higher than adult populations. Although regulations try to prevent food originated BPA exposure of infants and children, BPA's extensive use in other commodities places humans in an environment with abundant levels of this chemical. Increasing knowledge of BPA-related toxicity in children clarifies that exposure to BPA leads to health risks especially in sensitive developmental periods. The determination of the exposure to BPA through biomonitoring specifically in children is very important for public health in all countries. This first study of biomonitoring in preschool children from Turkey should be followed by other studies from large communities from various provinces. Future studies should concentrate on children highly exposed to BPA during critical periods of their life span for understanding future health risks of BPA exposure. **Children, in this study, are more likely to be exposed to different chemicals, including BPA due being at the age of preschooling and therefore spending most of their times in preschools. It is believed that results of this study would facilitate bases for future chemical compounds trainings, to be delivered for preschools**

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Ethics Ethics Committee Approval:

The study was approved by the Ethics Commission of the Mersin University Clinical Research Ethical Committees (Protocol number: 12.02.2015/37).

Informed Consent: Written informed consent was obtained from the childrens' parents.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions Concept: İsmet Çok, Design: İsmet Çok, Sample Collection and Analysis: Dilek Battal, Özlem Toprak İkidağ, Ayça Aktaş, **Analysis or Interpretation**: Özlem Toprak İkidağ, Dilek Battal, İsmet Çok, Literature Search. Özlem Toprak İkidağ, Writing: İsmet Çok

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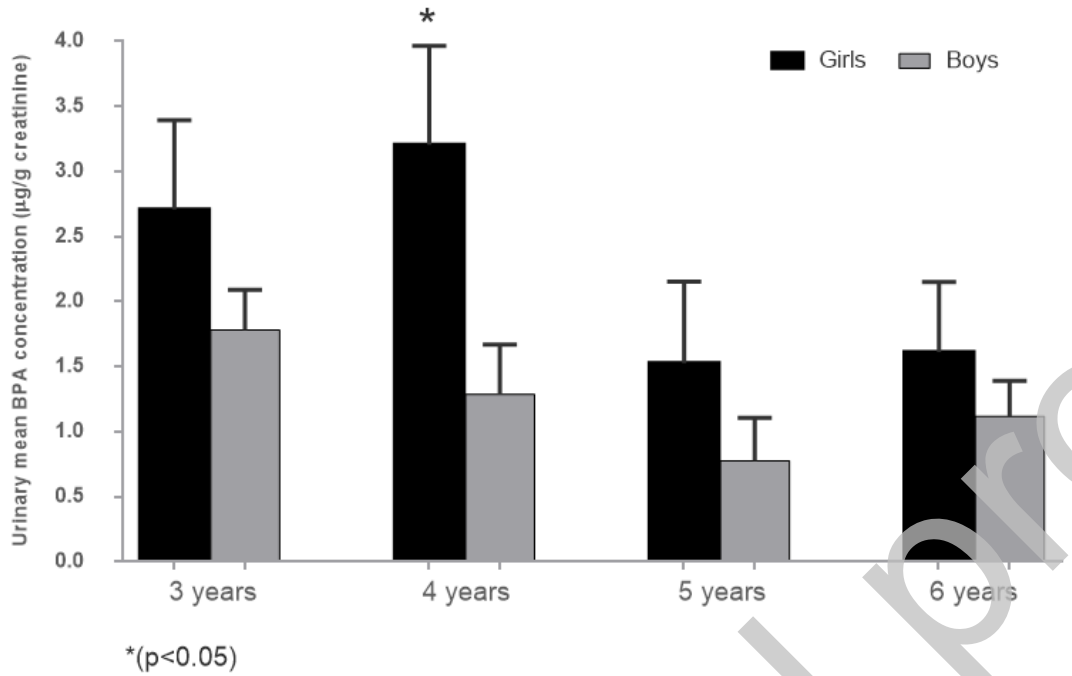

Figure 1 Urinary mean BPA values obtained in age groups (µg/g creatinine).

Table 1. Population characteristics

		Mean (Min-Max)
AGE (year)	Female (n=55)	4.42 (3-6)
	Male (n=70)	4.56 (3-6)
TOTAL	n=125	4.50 (3-6)
HEIGHT (cm)	Female	107 (78-126)
	Male	109 (85-138)
WEIGHT (kg)	Female	18.28 (11-29)
	Male	19.30 (12-36)
BMI (kg/m^2)	Female	16.00 (12.15-23.99)
	Male	16.21(11.81-26.67)

Table 2. Urinary concentration of BPA in Turkish preschool children (µg/g creatinine).

TOTAL BPA	n	>LOQ[a] (%)	Min-max	Mean±SD	Median	GM	95% CI	p[b]
Males	70	77	LOQ- 9.34	1,26±2,16	0.50	0.81	0.51-1.77	
Females	55	76	LOQ-18.36	2.24±2,24	0.65	0.98	0.83-3.57	0,2022
∑	125	77	LOQ-18.36	1,79±3,74	0.60	1.05	1.13-2.45	

[a] LOQ:limit of quantification, 0.33 ng/ml; [b]Mann-Whitney U test; GM:geometric mean; 95% CI: 95% confidence interval;

Table 3. Urinary BPA values related to age groups in **females and males** (µg/g creatinine)

Age	N	FEMALES			MALES			p[a]
		N	Median	Max	N	Median	Max	
<4 years	70	32	1.33	18.36	38	0.43	9.34	<0.005
>4 years	55	23	0.30	16.6	32	0.56	8.89	>0.05

[a]Mann-Whitney U test

Table 4 . Estimated daily intake of BPA in Turkish preschool children (µg/kg bw/day).

	N	GM (95% Cl)	95th
Males	70	0.031 (0.028-0.034)	0.189
Females	55	0.042 (0.037-0.046)	0.245
Total	125	0.035 (0.030-0.039)	0.206
3 years	35	0.038 (0.033-0.041)	0.236
4 years	35	0.046 (0.037-0.051)	0.264
5 years	25	0.024 (0.020-0.026)	0.171
6 years	30	0.036 (0.034-0.040)	0.242

GM:geometric mean; 95% CI: 95% confidence interval;

Table 5. Urinary Bisphenol A (BPA) concentrations in children from different countries.

COUNTRY	YEAR	N	AGES	Urinary BPA Concentrations	References
Canada	2007-2009 2009-2011	590	6-8	1.8 µg/L GM	6
China	2014- 2017	253 Control 215 ADHD	6-12	Control (6-9) 1.58 µg/L GM ADHD (6-9) 3.44 µg/L GM	30
Germany	2007-2008	137 145	3-5 6-8	3.55 µg/L GM 2.72 µg/L GM	25
South Korea	2001-2006 2016	164	3-5 7-9	0.76 µg/g creatinine Median 0.61 µg/g creatinine Median	31
USA	2001-2010	408 401 318	3 5 7	7.4 µg/L Mean 5.4 µg/L Mean 5.8 µg/L Mean	32
USA	2004 - 2014	-Non-Hispanic White: 200 - Non-Hispanic Black : 96	1-8	(White) 2.7 µg/L GM (Black) 3.2 µg/L GM	33
Spain	2011 - 2012	60 59	5-8 9-11	2.33 µg /g creatinine GM 1.72 µg /g creatinine GM	34
Avustralia	2012- 2013	64 Boys 36 Girls	2-3	(Boys) 3.09 µg/L GM (Girls) 2.17 µg/L GM 2.72 µg/L GM	35
Portugal	2014-2015	70	4-11	1.87 µg /g creatinine Median	36
Turkey	2015-2016	70 Boys 55 Girls	3-6	(Boys) 0.81 µg /g creatinine GM (Girls) 0.98 µg /g creatinine GM	Çok et al. (this study)

Table 6. BPA-specific questionnaire data of the study population, mean±S.D.

	N	% N	BPA-TOTAL (µg/g creatinine)
FAST FOOD CONSUMPTION HABIT			
None	11	8.8	1.63±2.63
1 meal/month	67	53.6	1.94±3.77
1-3 meals/month	39	31.2	2.04±3.83
3-5 meals/month	8	6.4	1.63±2.01
PLASTIC COATED FOOD PRODUCT PURCHASE			
Never	27	21.6	2.44± 0.86
Sometimes	86	68.8	1.47±2.76
Always	12	9.6	2.92±3.83
HEATING FOOD WITHIN A PLASTIC CUP IN MICROWAVE			
Yes	10	8	2.88±5.58
No	115	92	1.72±3.05
CONSIDERING INSTRUCTIONS FOR PACKAGING AND LABELLING			
Sometimes	40	32.0	2.11±3.63
Always	82	65.6	1.70±3.19
Never	3	2.40	1.99±2.87
EXISTANCE OF WHITE DENTAL FILLING			
Yes	11	8.80	0.62±0.91
No	114	91.2	1.93±3.43
QUALITY OF THE PURCHASED PLASTIC			
I wouldn't purchase low priced products	25	20.0	2.33±4.71
I wouldn't mind	24	19.2	1.22±1.46
I would purchase well-known brands	32	25.6	2.65±4.31
FEATURE OF THE PURCHASED TOY			
Mostly metal toys	11	8.8	2.22±3.43
Mostly wooden toys	8	6.4	1.70±1.12
Mostly plastic toys	106	84.8	1.82±3.45
WASHING THE PLASTIC FOOD CONTAINERS IN A DISHWASHER			
Yes	55	44.0	2.78±3.31
No	75	56.0	1.94±4.04
CANNED FOOD CONSUMPTION			
Yes	10	8.0	1.70±3.19

No	115	92.0	2.94±3.77
CANNED BEVERAGES CONSUMPTION			
Yes	78	62.4	2.74± 1.80
No	47	37.6	1.67±2.87
STORAGE OF FOOD IN PVC CONTAINERS			
Sometimes	33	26.4	2.41±3.13
Always	92	73.6	2.65±2.96
Never	-	-	-